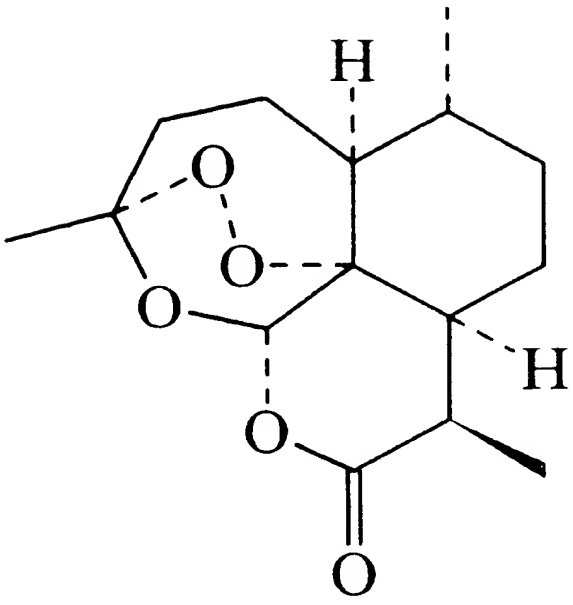


FIG. 1

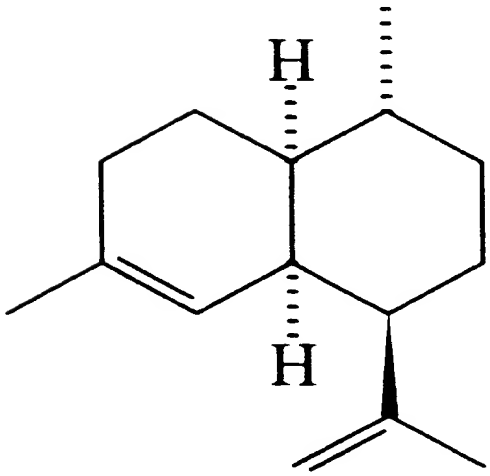


FIG. 4

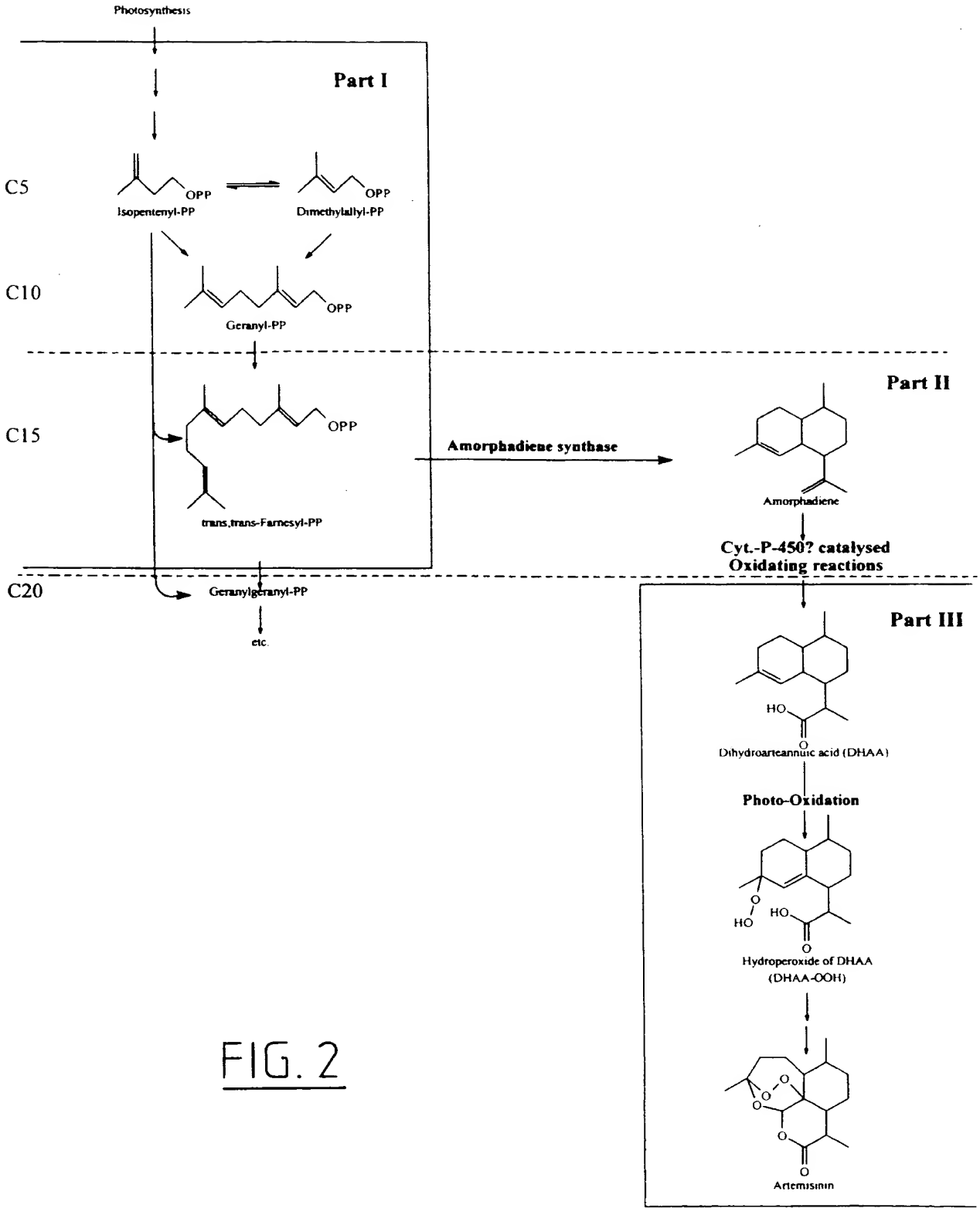
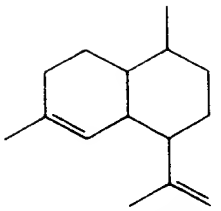

Photosynthesis
Part I
C5
Isopentenyl-PP
Dimethylallyl-PP
C10
Geranyl-PP
C15
trans,trans-Farnesyl-PP
C20
Geranylgeranyl-PP
etc.
Amorphadiene synthase
Part II
Amorphadiene
Cyt.-P-450? catalysed
Oxidating reactions
Part III
Dihydroarteannuic acid (DHAA)
Photo-Oxidation
Hydroperoxide of DHAA
(DHAA-OOH)
Artemisinin

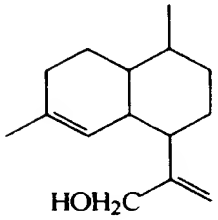
FIG. 2



Amorphadiene

NADPH
NADP+

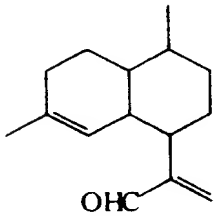
Amorphadiene hydroxylase (cyt P-450?)



Amorphadienol

NADPH
NADP+

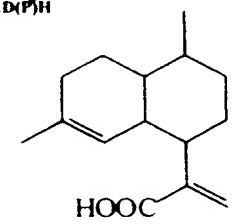
Amorphadienol oxygenase (cyt P-450?)



Amorphadienal

NADPH
NADP+
or
NAD(P)+
NAD(P)H

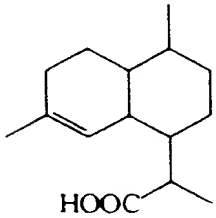
Amorphadienal hydroxylase (cyt P-450?) or Amorphadienal dehydrogenase



Arteannuic acid

NADPH
NADP+

Arteannuic acid reductase (enoaat reductase)



Dihydroarteannuic acid

FIG. 3

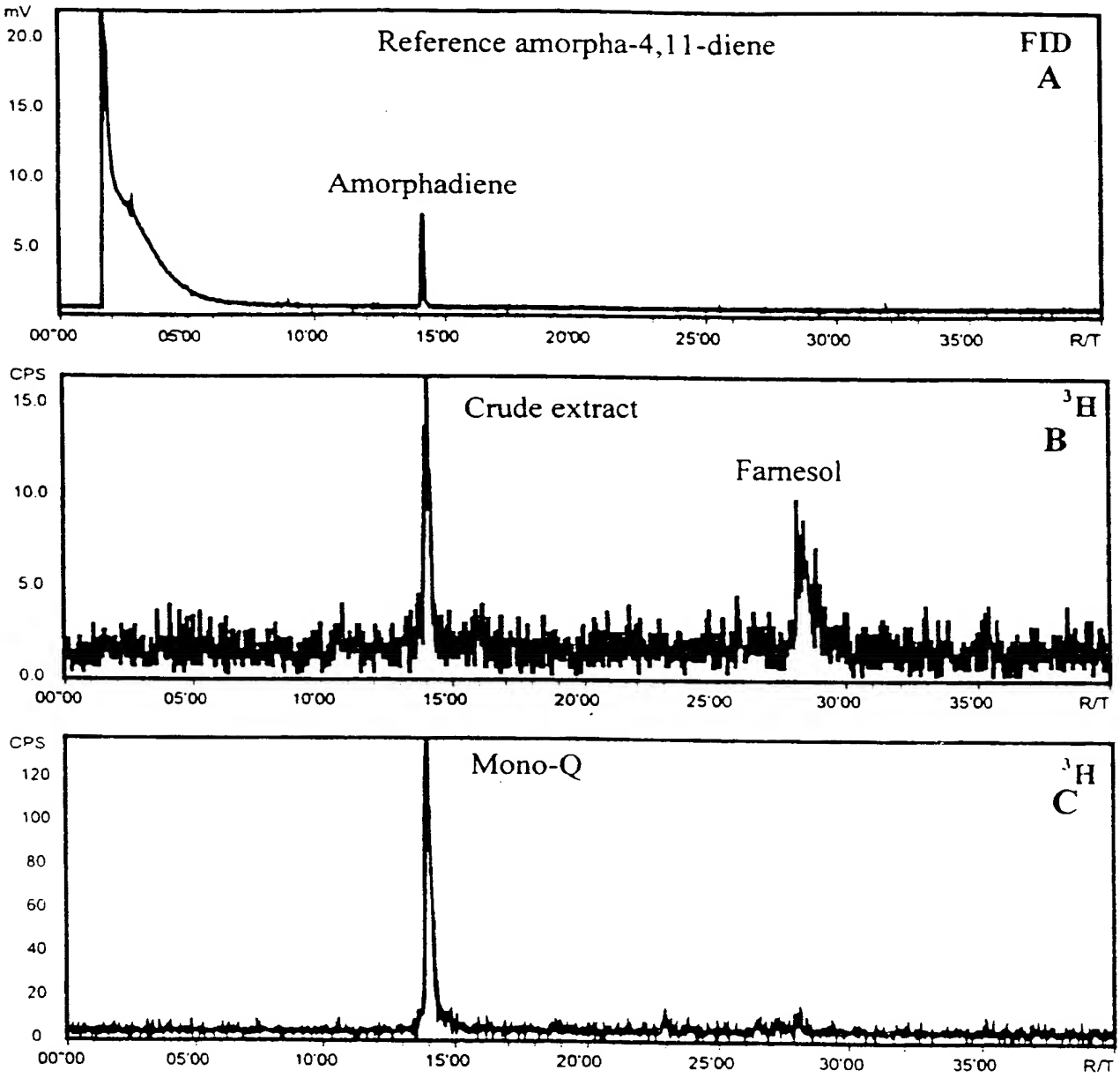

mV
20.0
15.0
10.0
5.0
Reference amorpha-4,11-diene
FID
A
Amorphadiene
00'00
05'00
10'00
15'00
20'00
25'00
30'00
35'00
R/T
CPS
15.0
10.0
5.0
0.0
Crude extract
^{3}H
B
Farnesol
CPS
120
100
80
60
40
20
0
Mono-Q
^{3}H
C

FIG. 5

Library Searched : C:\DATABASE\WITLOF.L
Quality : 99
ID : amorpha-4,11-dieen

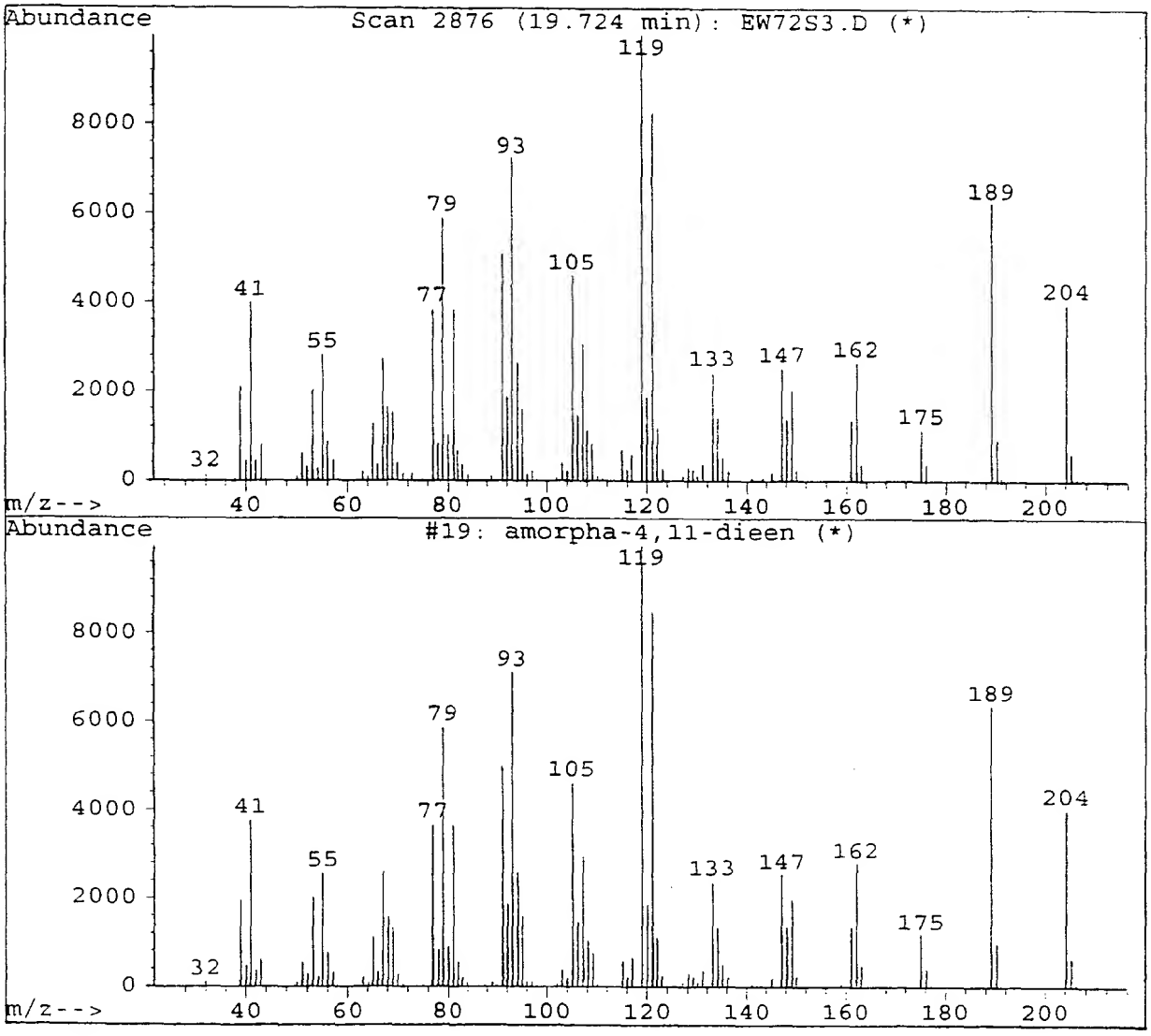


FIG. 6

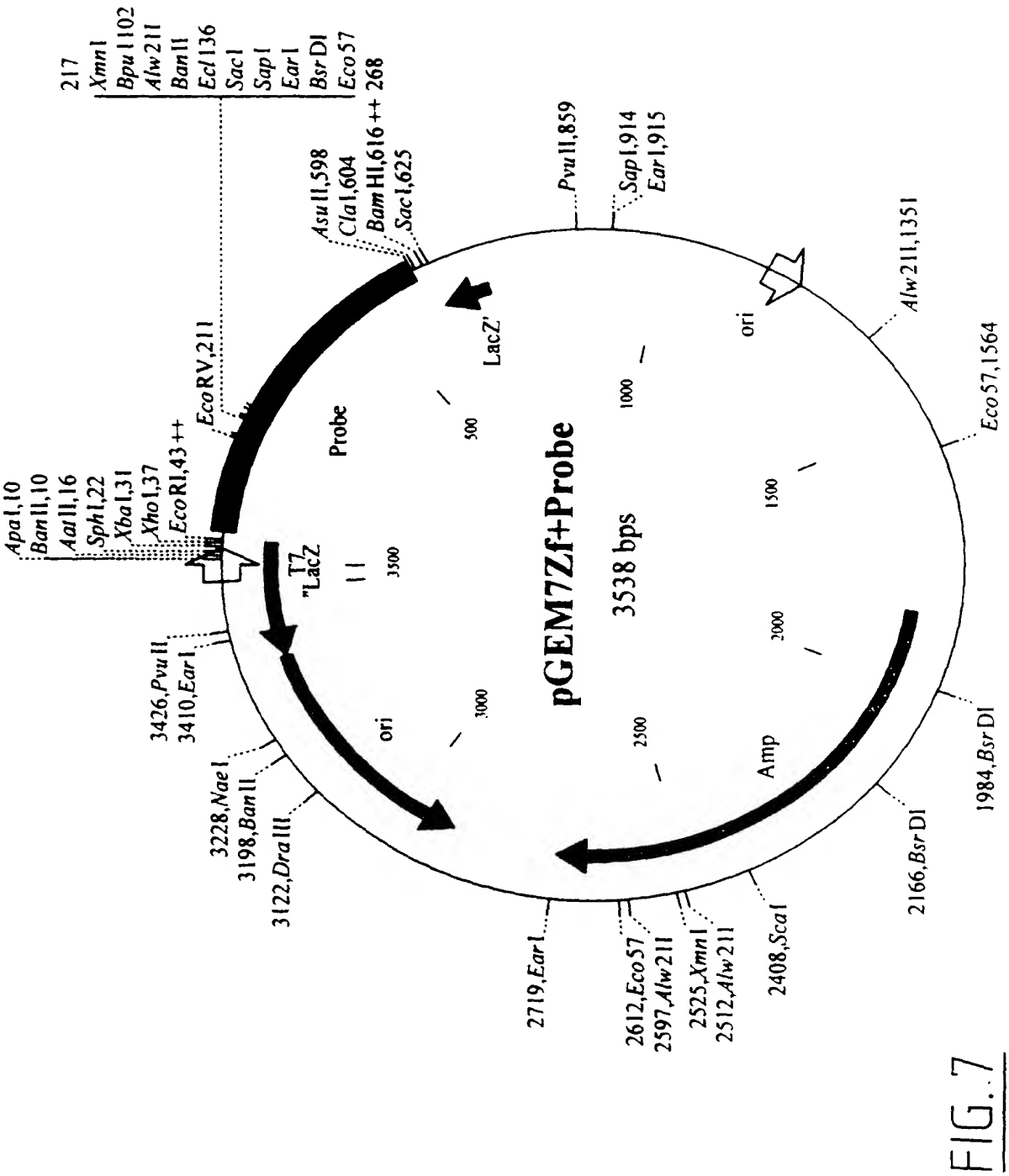

pGEM7Zf+Probe
3538 bps
Probe
LacZ'
T7
'LacZ
ori
Amp
ori
500
1000
1500
2000
2500
3000
3500
217
Xmn I
Bpu 1102
Alw 211
Ban II
Ecl 136
Sac I
Sap I
Ear I
Bsr DI
Eco 57
Asu II,598
Cla I,604
BamHI,616 ++ 268
Sac I,625
Pvu II,859
Sap I,914
Ear I,915
Alw 211,1351
Eco 57,1564
1984,Bsr DI
2166,Bsr DI
2408,Sca I
2512,Alw 211
2525,Xmn I
2597,Alw 211
2612,Eco 57
2719,Ear I
3122,Dra III
3198,Ban II
3228,Nae I
3410,Ear I
3426,Pvu II
Eco RV,211
Eco RI,43 ++
Xho I,37
Xba I,31
Sph I,22
Aat II,16
Ban II,10
Apa I,10

FIG. 7

```
                    Primer A
27   gat gag aat ggg aaa ttt aag gaa tcg tta gct aat gat gtt gaa ggt ttg
      D   E   N   G   K   F   K   E   S   L   A   N   D   V   E   G   L

78   ctt gag ttg tac gaa gca act tct atg agg gta cct ggg gag att ata tta
      L   E   L   Y   E   A   T   S   M   R   V   P   G   E   I   I   L

129  gaa gat gct ctt ggt ttt aca cga tct cgt ctt agc att atg aca aaa gat
      E   D   A   L   G   F   T   R   S   R   L   S   I   M   T   K   D

180  gct ttt tct aca aac ccc gct ctt ttt acc gaa ata caa cgg gca cta aag
      A   F   S   T   N   P   A   L   F   T   E   I   Q   R   A   L   K

231  caa ccc ctt tgg aaa agg ttg cca aga ata gag gcg gcg cag tac att cct
      Q   P   L   W   K   R   L   P   R   I   E   A   A   Q   Y   I   P

282  ttc tat caa caa caa gat tct cat aac aag act tta ctt aaa ctt gct aag
      F   Y   Q   Q   Q   D   S   H   N   K   T   L   L   K   L   A   K

333  tta gag ttc aat ttg ctt cag tca ttg cac aag gaa gag ctc agc cat gtg
      L   E   F   N   L   L   Q   S   L   H   K   E   E   L   S   H   V

384  tgc aaa tgg tgg aaa gct ttc gat atc aag aag aac gca cct tgt tta aga
      C   K   W   W   K   A   F   D   I   K   K   N   A   P   C   L   R

435  gat aga att gtt gaa tgc tac ttt tgg gga cta ggt tca ggc tat gag cca
      D   R   I   V   E   C   Y   F   W   G   L   G   S   G   Y   E   P

486  cag tat tcc cgg gct aga gtt ttc ttc aca aaa gct gtt gct gtt ata act
      Q   Y   S   R   A   R   V   F   F   T   K   A   V   A   V   I   T
                           Primer B
537  ctt ata gac gac acc ttc gac gct acg g
      L   I   D   D   T   F   D   A   T
```

FIG. 8

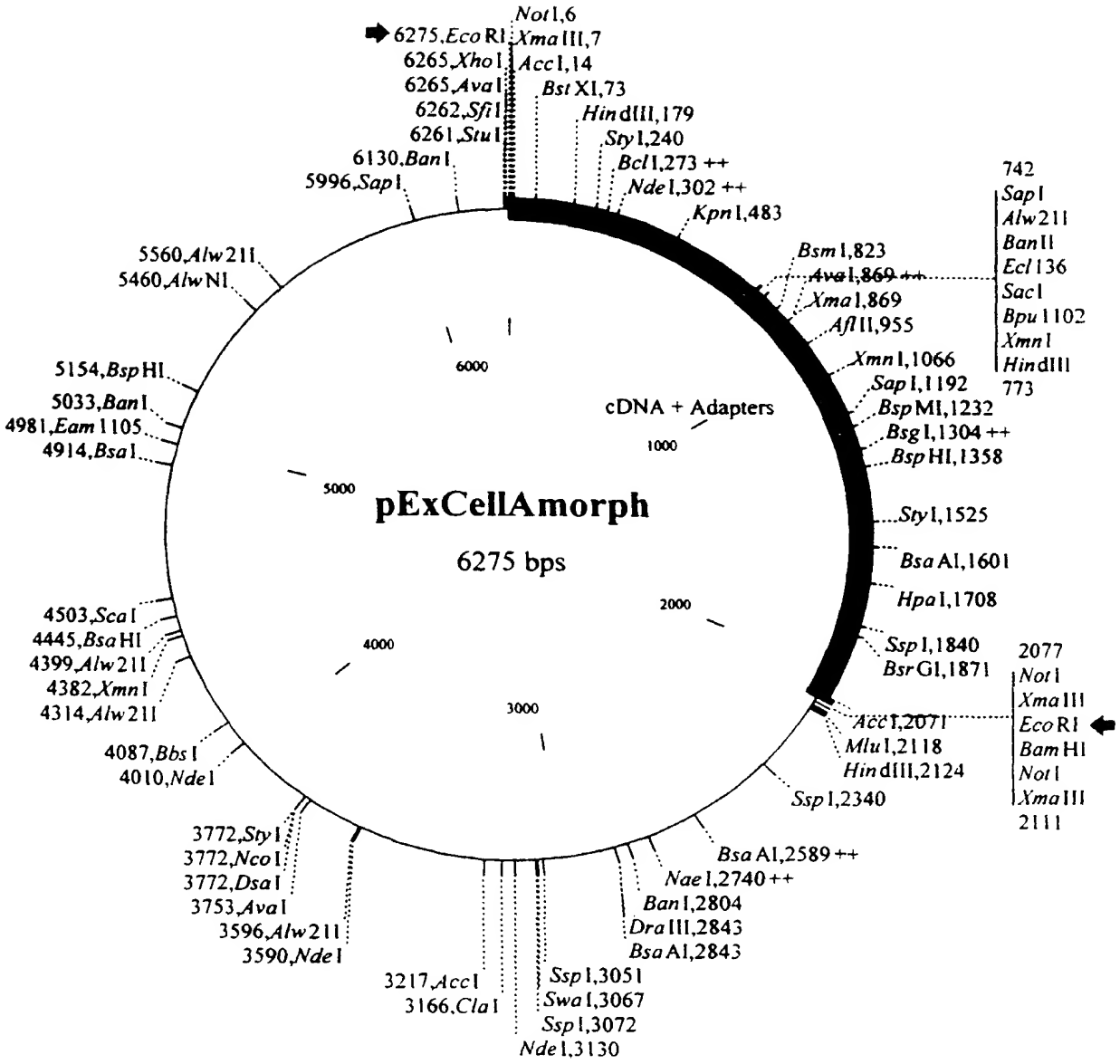

pExCellAmorph
6275 bps
cDNA + Adapters
1000
2000
3000
4000
5000
6000
Not I,6
Xma III,7
Acc I,14
Bst XI,73
Hin dIII,179
Sty I,240
Bcl I,273 ++
Nde I,302 ++
Kpn I,483
Bsm I,823
Ava I,869 ++
Xma I,869
Afl II,955
Xmn I,1066
Sap I,1192
Bsp MI,1232
Bsg I,1304 ++
Bsp HI,1358
Sty I,1525
Bsa AI,1601
Hpa I,1708
Ssp I,1840
Bsr GI,1871
Acc I,2071
Mlu I,2118
Hin dIII,2124
Ssp I,2340
Bsa AI,2589 ++
Nae I,2740 ++
Ban I,2804
Dra III,2843
Bsa AI,2843
Ssp I,3051
Swa I,3067
Ssp I,3072
Nde I,3130
3217,Acc I
3166,Cla I
3772,Sty I
3772,Nco I
3772,Dsa I
3753,Ava I
3596,Alw 21I
3590,Nde I
4087,Bbs I
4010,Nde I
4503,Sca I
4445,Bsa HI
4399,Alw 21I
4382,Xmn I
4314,Alw 21I
5154,Bsp HI
5033,Ban I
4981,Eam 1105
4914,Bsa I
5560,Alw 21I
5460,Alw NI
5996,Sap I
6130,Ban I
6262,Sfi I
6261,Stu I
6265,Ava I
6265,Xho I
6275,Eco RI
742
Sap I
Alw 21I
Ban II
Ecl 136
Sac I
Bpu 1102
Xmn I
Hin dIII
773
2077
Not I
Xma III
Eco RI
Bam HI
Not I
Xma III
2111

FIG. 9

*Eco*R I (*Not* I) Adapter

```
 1  aattcgcggc cgcgtcgaca aatcatgtca cttacagaag aaaaacctat
      N  S  R    P  R  R    Q  I  M  S    L  T  E    E  K  P
 ← EcoR I      Not I

51  tcgccccatt gccaactttc ctccaagcat ttggggagat cagtttctca
      I  R  P  I    A  N  F    P  P  S    I  W  G  D    Q  F  L

101 tctatcaaaa gcaagtagag caaggggtgg aacagatagt gaatgattta
      I  Y  Q    K  Q  V  E    Q  G  V    E  Q  I    V  N  D  L

151 aaaaaagaag tgcggcaact actaaaagaa gctttggata ttcctatgaa
      K  K  E    V  R  Q    L  L  K  E    A  L  D    I  P  M

201 acatgccaat ttgttgaagc tgattgatga aattcaacgc cttggaatac
      K  H  A  N    L  L  K    L  I  D    E  I  Q  R    L  G  I

251 cgtatcactt tgaacgggag attgatcatg cattgcaatg tatttatgaa
      P  Y  H    F  E  R  E    I  D  H    A  L  Q    C  I  Y  E

301 acatatggtg ataactggaa tggtgaccgc tcttccttat ggttccgtct
      T  Y  G    D  N  W    N  G  D  R    S  S  L    W  F  R

351 tatgcgaaag caaggatatt atgttacatg tgatgttttc aataactata
      L  M  R  K    Q  G  Y    Y  V  T    C  D  V  F    N  N  Y

401 aagacaaaaa tggagcgttc aagcaatcgt tagctaatga tgttgaaggt
      K  D  K    N  G  A  F    K  Q  S    L  A  N    D  V  E  G

451 ttgcttgagt tgtacgaagc aacttctatg agggtacctg gggagattat
      L  L  E    L  Y  E    A  T  S  M    R  V  P    G  E  I

501 attagaagat gctcttggtt ttacacgatc tcgtcttagc attatgacaa
      I  L  E  D    A  L  G    F  T  R    S  R  L  S    I  M  T

551 aagatgcttt ttctacaaac cccgctcttt ttaccgaaat acaacgggca
      K  D  A    F  S  T  N    P  A  L    F  T  E    I  Q  R  A

601 ctaaagcaac ccctttggaa aaggttgcca agaatagagg cggcgcagta
      L  K  Q    P  L  W    K  R  L  P    R  I  E    A  A  Q

651 cattcctttc tatcaacaac aagattctca taacaagact ttacttaaac
      Y  I  P  F    Y  Q  Q    Q  D  S    H  N  K  T    L  L  K

701 ttgctaagtt agagttcaat ttgcttcagt cattgcacaa ggaagagctc
      L  A  K    L  E  F  N    L  L  Q    S  L  H    K  E  E  L

751 agccatgtgt gcaaatggtg gaaagctttc gatatcaaga agaacgcacc
      S  H  V    C  K  W    W  K  A  F    D  I  K    K  N  A

801 ttgtttaaga gatagaattg ttgaatgcta cttttgggga ctaggttcag
      P  C  L  R    D  R  I    V  E  C    Y  F  W  G    L  G  S

851 gctatgagcc acagtattcc cgggctagag ttttcttcac aaaagctgtt
      G  Y  E    P  Q  Y  S    R  A  R    V  F  F    T  K  A  V
```

FIG. 10-1

```
901  gctgttataa ctcttataga tgacacttat gatgcgtatg gtacttatga
       A  V  I    T  L  I    D  D  T  Y    D  A  Y    G  T  Y

951  agaacttaag atctttactg aagctgttga aaggtggtca attacatgct
       E  E  L  K    I  F  T    E  A  V    E  R  W  S    I  T  C

1001 tagacacact tccagaatac atgaaaccga tatacaaatt attcatggat
       L  D  T    L  P  E  Y    M  K  P    I  Y  K    L  F  M  D

1051 acatacacag aaatggaaga atttcttgca aaggagggaa gaacagatct
       T  Y  T    E  M  E    E  F  L  A    K  E  G    R  T  D

1101 atttaactgc ggcaaagaat ttgtgaaaga gtttgttaga aacctgatgg
       L  F  N  C    G  K  E    F  V  K    E  F  V  R    N  L  M

1151 ttgaagcaaa atgggcaaat gagggacaca taccaaccac tgaagagcat
       V  E  A    K  W  A  N    E  G  H    I  P  T    T  E  E  H

1201 gatccagttg taatcattac tggcggtgct aacctgctta caacaacttg
       D  P  V    V  I  I    T  G  G  A    N  L  L    T  T  T

1251 ttatcttggc atgagtgata tattcacaaa agagtctgtc gaatgggctg
       C  Y  L  G    M  S  D    I  F  T    K  E  S  V    E  W  A

1301 tctctgcacc tcctcttttt agatactcag gtatacttgg tcgacgccta
       V  S  A    P  P  L  F    R  Y  S    G  I  L    G  R  R  L

1351 aatgatctca tgacccacaa ggccgagcaa gaaagaaaac atagttcatc
       N  D  L    M  T  H    K  A  E  Q    E  R  K    H  S  S

1401 gagccttgaa agttatatga aggaatataa tgtcaatgag gagtatgccc
       S  S  L  E    S  Y  M    K  E  Y    N  V  N  E    E  Y  A

1451 aaaccttgat ttacaaggaa gtagaagatg tgtggaaaga tataaaccga
       Q  T  L    I  Y  K  E    V  E  D    V  W  K    D  I  N  R

1501 gagtacctca caactaaaaa cattccaagg ccgttattga tggctgtgat
       E  Y  L    T  T  K    N  I  P  R    P  L  L    M  A  V

1551 ctatttgtgc cagtttcttg aagttcaata tgcaggaaag gataacttca
       I  Y  L  C    Q  F  L    E  V  Q    Y  A  G  K    D  N  F

1601 cacgtatggg agacgaatac aaacatctca taaagtctct actcgtttat
       T  R  M    G  D  E  Y    K  H  L    I  K  S    L  L  V  Y

1651 cctatgagta tatgactacc aatccttcgt gcatagccta tcaattatat
       P  M  S    I  -  L    P  I  L  R    A  -  P    I  N  Y

1701 tgaaagggtt aactatgcac gtctctatgg agagaatttc tcaagctatt
       I  E  R  V    N  Y  A    R  L  Y    G  E  N  F    S  S  Y
```

FIG. 10-2

```
1751  tggtgtttct tgctggcaat aataaatcag acgcataaaa ttgtattgaa
        L  V  F    L  A  G  N    N  K  S    D  A  -    N  C  I  E

1801  ctatatgccg atagctattt aaagttatta tacaactaaa atattcaaca
        L  Y  A    D  S  Y    L  K  L  L    Y  N  -    N  I  Q

1851  atggtattat acttttactt tgtacaaaag caaaagtaca ctactgttat
        Q  W  Y  Y    T  F  T    L  Y  K    S  K  S  T    L  L  L

1901  gtaacatttt agttctatga tactttagtt acgaatcggc ttatatacat
        C  N  I    L  V  L  -    Y  F  S    Y  E  S    A  Y  I  H

1951  tgatacactt ttatgcagaa aaccctagta aataaaaagt cgatatcttg
        -  Y  T    F  M  Q    K  T  L  V    N  K  K    S  I  S

2001  tactacacat atcgcacgaa tttccgtttg ccgtttgtat tttacgatat
        C  T  T  H    I  A  R    I  S  V    C  R  L  Y    F  T  I

2051  gttatttaat gaatatgttt catgtggttg ttgcttaaaa aaaaagtcga
        C  Y  L    M  N  M  F    H  V  V    V  A  -    K  K  S  R
      [ Not I ]  [ EcoR I ➡
2101  cgcggccgcg aa
        R  G  R ]  E
```

*Eco*R I (*Not* I) Adapter

FIG. 10-3

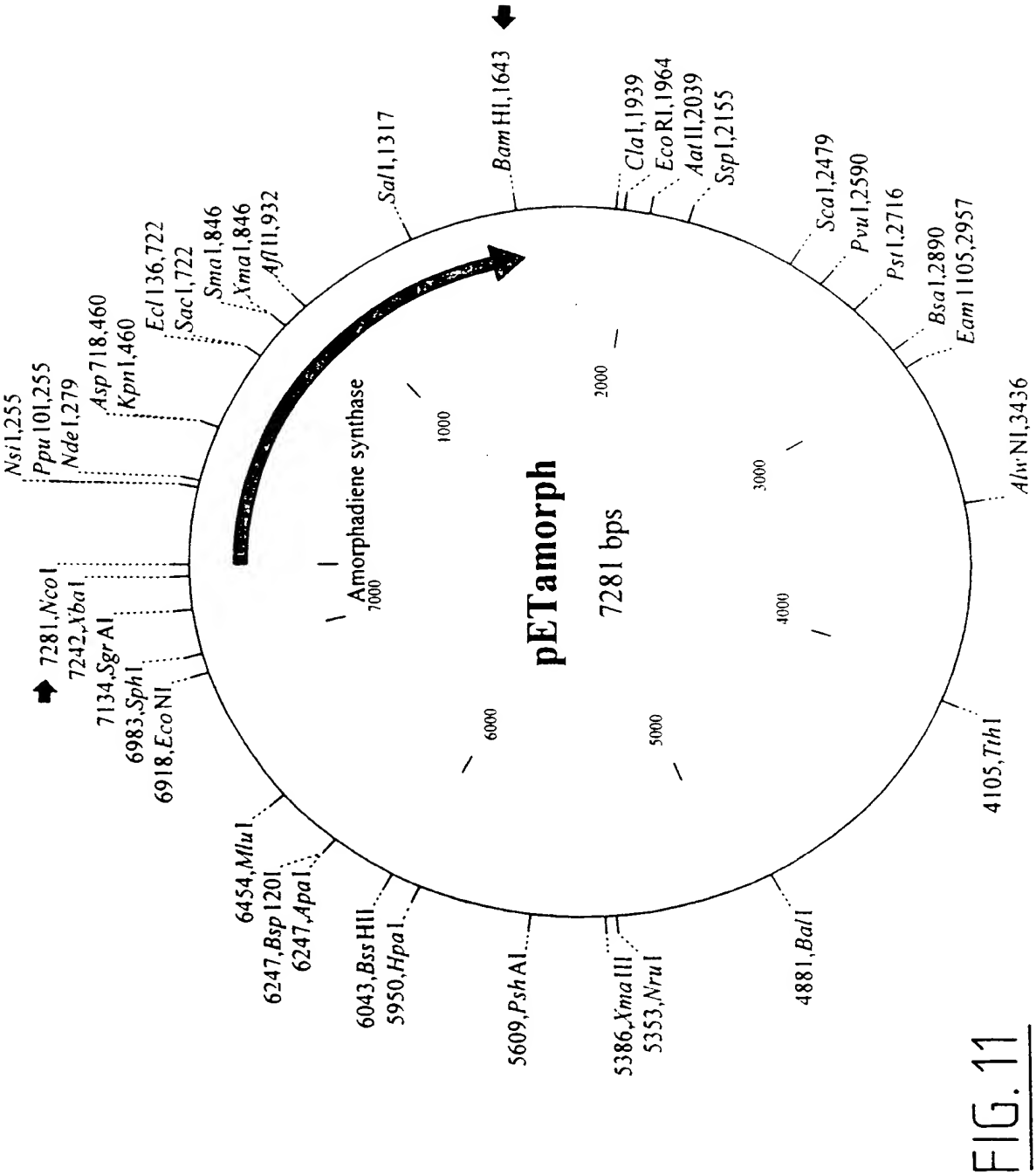

pETamorph
7281 bps
Amorphadiene synthase
1000
2000
3000
4000
5000
6000
7000
BamHI,1643
ClaI,1939
EcoRI,1964
AatII,2039
SspI,2155
ScaI,2479
PvuI,2590
PstI,2716
BsaI,2890
EamI105,2957
AlwNI,3436
4105,TthI
4881,BalI
5353,NruI
5386,XmaIII
5609,PshAI
5950,HpaI
6043,BssHII
6247,ApaI
6247,BspI201
6454,MluI
6918,EcoNI
6983,SphI
7134,SgrAI
7242,XbaI
7281,NcoI
Nsi1,255
Ppu101,255
NdeI,279
Asp718,460
KpnI,460
Ecl136,722
SacI,722
SmaI,846
XmaI,846
AflII,932
SalI,1317

FIG. 11

```
        Nco I
      ccatggcact tacagaagaa aaacctattc gccccattgc caactttcct
7281    T M A   L T E E   K P I   R P I   A N F P
        Start codon
      ccaagcattt ggggagatca gtttctcatc tatcaaaagc aagtagagca
  50    P S I   W G D   Q F L I   Y Q K   Q V E
      aggggtggaa cagatagtga atgatttaaa aaaagaagtg cggcaactac
 100    Q G V E   Q I V   N D L   K K E V   R Q L
      taaaagaagc tttggatatt cctatgaaac atgccaattt gttgaagctg
 150    L K E   A L D I   P M K   H A N   L L K L
      attgatgaaa ttcaacgcct tggaataccg tatcactttg aacgggagat
 200    I D E   I Q R   L G I P   Y H F   E R E
      tgatcatgca ttgcaatgta tttatgaaac atatggtgat aactggaatg
 250    I D H A   L Q C   I Y E   T Y G D   N W N
      gtgaccgctc ttccttatgg ttccgtctta tgcgaaagca aggatattat
 300    G D R   S S L W   F R L   M R K   Q G Y Y
      gttacatgtg atgttttcaa taactataaa gacaaaaatg gagcgttcaa
 350    V T C   D V F   N N Y K   D K N   G A F
      gcaatcgtta gctaatgatg ttgaaggttt gcttgagttg tacgaagcaa
 400    K Q S L   A N D   V E G   L L E L   Y E A
      cttctatgag ggtacctggg gagattatat tagaagatgc tcttggtttt
 450    T S M   R V P G   E I I   L E D   A L G F
      acacgatctc gtcttagcat tatgacaaaa gatgcttttt ctacaaaccc
 500    T R S   R L S   I M T K   D A F   S T N
      cgctcttttt accgaaatac aacgggcact aaagcaaccc ctttggaaaa
 550    P A L F   T E I   Q R A   L K Q P   L W K
      ggttgccaag aatagaggcg gcgcagtaca ttcctttcta tcaacaacaa
 600    R L P   R I E A   A Q Y   I P F   Y Q Q Q
      gattctcata acaagacttt acttaaactt gctaagttag agttcaattt
 650    D S H   N K T   L L K L   A K L   E F N
      gcttcagtca ttgcacaagg aagagctcag ccatgtgtgc aaatggtgga
 700    L L Q S   L H K   E E L   S H V C   K W W
      aagctttcga tatcaagaag aacgcacctt gtttaagaga tagaattgtt
 750    K A F   D I K K   N A P   C L R   D R I V
      gaatgctact tttggggact aggttcaggc tatgagccac agtattcccg
 800    E C Y   F W G   L G S G   Y E P   Q Y S
      ggctagagtt ttcttcacaa aagctgttgc tgttataact cttatagatg
 850    R A R V   F F T   K A V   A V I T   L I D
```

FIG. 12-1

```
900   acacttatga tgcgtatggt acttatgaag aacttaagat ctttactgaa
        D  T  Y   D  A  Y  G   T  Y  E   E  L  K   I  F  T  E

950   gctgttgaaa ggtggtcaat tacatgctta gacacacttc cagaatacat
        A  V  E   R  W  S   I  T  C  L   D  T  L   P  E  Y

1000  gaaaccgata tacaaattat tcatggatac atacacagaa atggaagaat
      M  K  P  I   Y  K  L   F  M  D   T  Y  T  E   M  E  E

1050  ttcttgcaaa ggagggaaga acagatctat ttaactgcgg caaagaattt
        F  L  A   K  E  G  R   T  D  L   F  N  C   G  K  E  F

1100  gtgaaagagt ttgttagaaa cctgatggtt gaagcaaaat gggcaaatga
        V  K  E   F  V  R   N  L  M  V   E  A  K   W  A  N

1150  gggacacata ccaaccactg aagagcatga tccagttgta atcattactg
      E  G  H  I   P  T  T   E  E  H   D  P  V  V   I  I  T

1200  gcggtgctaa cctgcttaca acaacttgtt atcttggcat gagtgatata
        G  G  A   N  L  L  T   T  T  C   Y  L  G   M  S  D  I

1250  ttcacaaaag agtctgtcga atgggctgtc tctgcacctc ctcttttag
        F  T  K   E  S  V   E  W  A  V   S  A  P   P  L  F

1300  atactcaggt atacttggtc gacgcctaaa tgatctcatg acccacaagg
      R  Y  S  G   I  L  G   R  R  L   N  D  L  M   T  H  K

1350  ccgagcaaga aagaaaacat agttcatcga gccttgaaag ttatatgaag
        A  E  Q   E  R  K  H   S  S  S   S  L  E   S  Y  M  K

1400  gaatataatg tcaatgagga gtatgcccaa accttgattt acaaggaagt
        E  Y  N   V  N  E   E  Y  A  Q   T  L  I   Y  K  E

1450  agaagatgtg tggaaagata taaaccgaga gtacctcaca actaaaaaca
      V  E  D  V   W  K  D   I  N  R   E  Y  L  T   T  K  N

1500  ttccaaggcc gttattgatg gctgtgatct atttgtgcca gtttcttgaa
        I  P  R   P  L  L  M   A  V  I   Y  L  C   Q  F  L  E

1550  gttcaatatg caggaaagga taacttcaca cgtatgggag acgaatacaa
        V  Q  Y   A  G  K   D  N  F  T   R  M  G   D  E  Y
                                                  [BamH I]
1600  acatctcata aagtctctac tcgtttatcc tatgagtata tgaggatcc
      K  H  L  I   K  S  L   L  V  Y   P  M  S  I   -  G  S
                                          Stop codon
```

FIG. 12-2

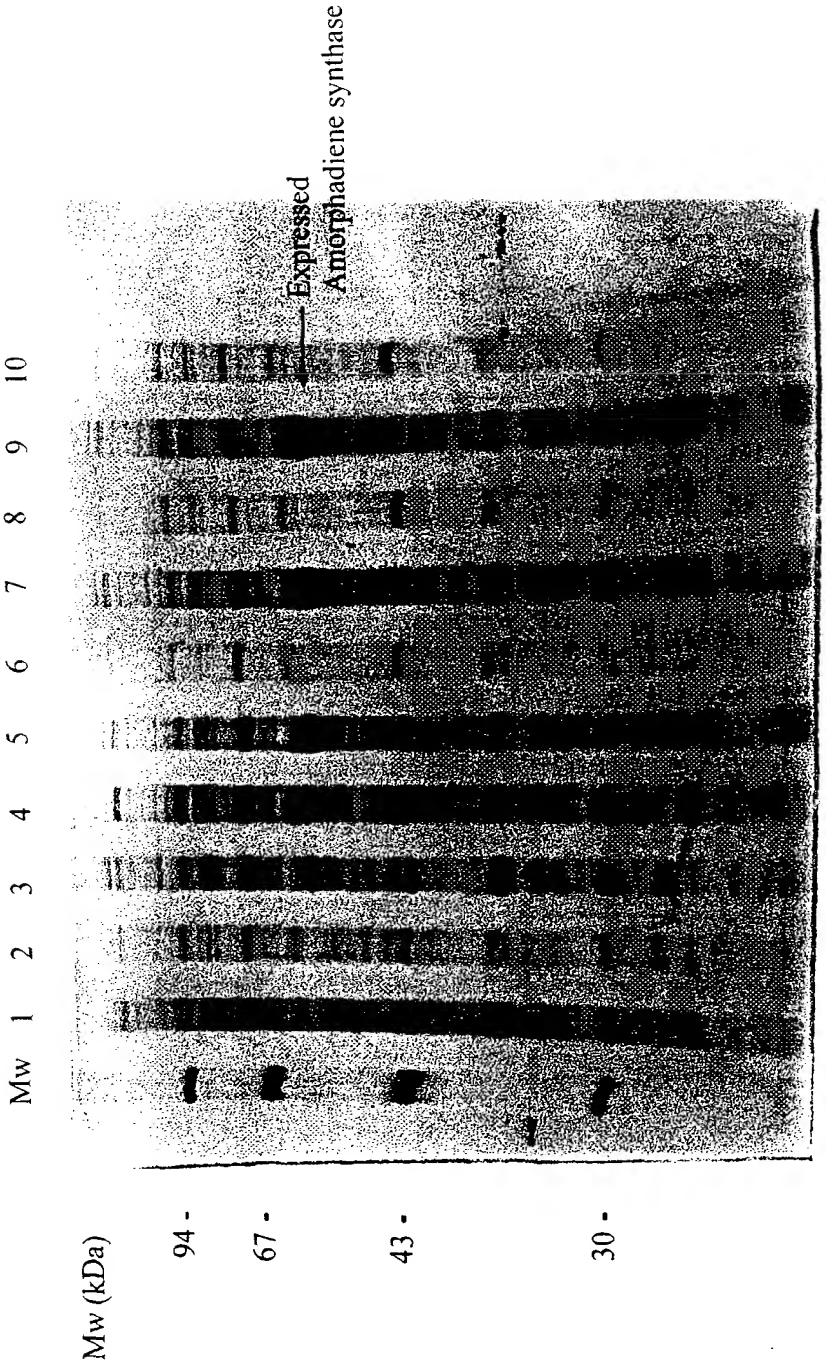

Expressed
Amorphadiene synthase
Mw 1 2 3 4 5 6 7 8 9 10
Mw (kDa)
94 -
67 -
43 -
30 -

FIG. 13

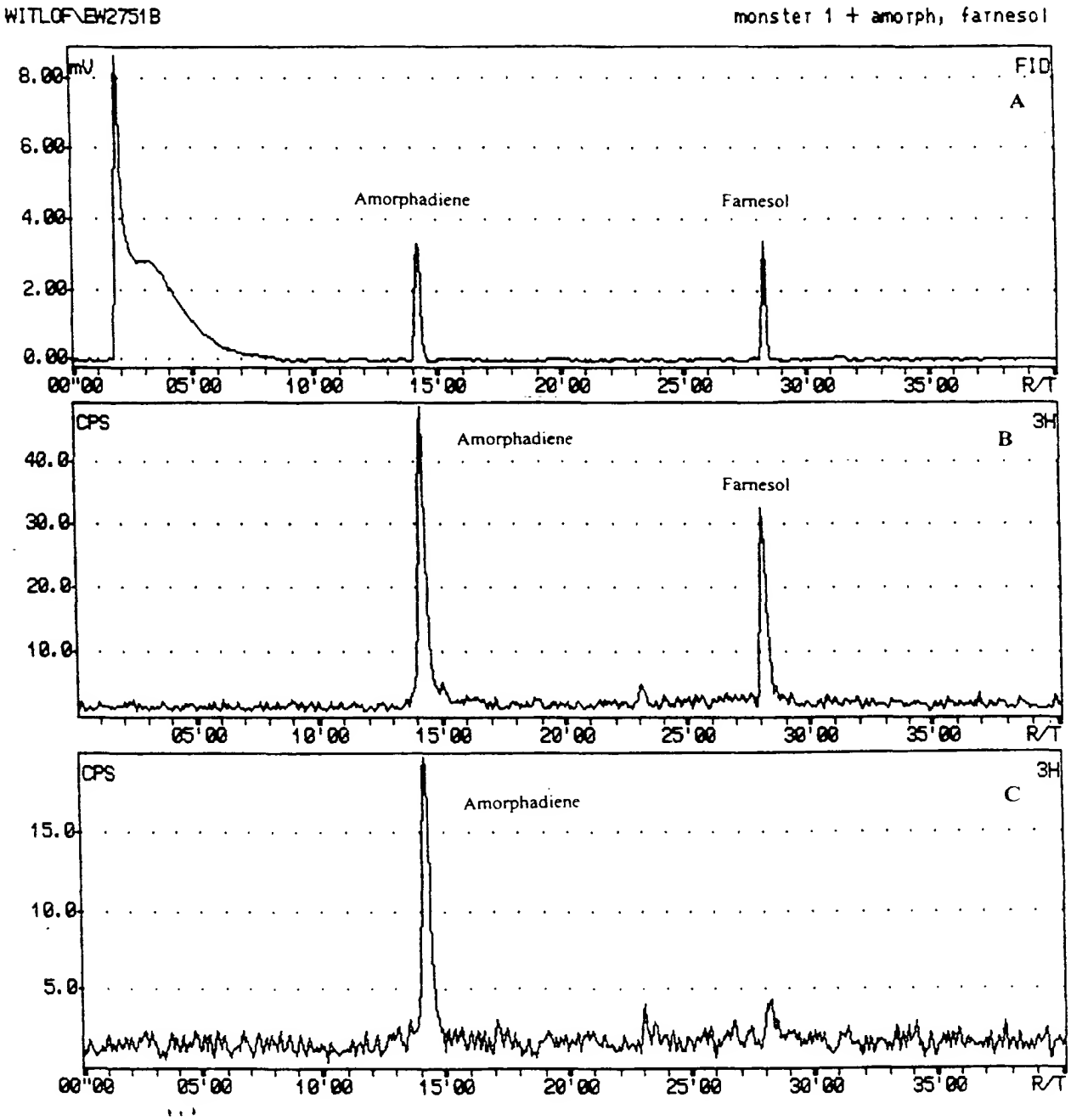

WITLOF\EW2751B
monster 1 + amorph, farnesol
mV
FID
A
8.00
6.00
4.00
2.00
0.00
Amorphadiene
Farnesol
00'00
05'00
10'00
15'00
20'00
25'00
30'00
35'00
R/T
CPS
3H
B
40.0
30.0
20.0
10.0
Amorphadiene
Farnesol
C
15.0
10.0
5.0

FIG. 14

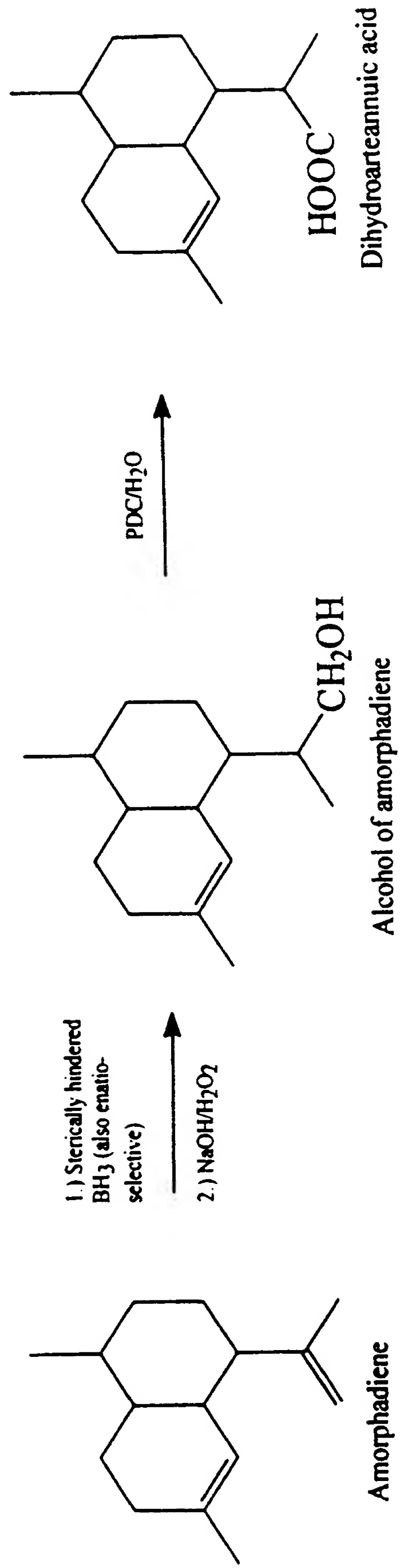

Amorphadiene
1.) Sterically hindered BH_3 (also enatio-selective)
2.) $NaOH/H_2O_2$
CH_2OH
Alcohol of amorphadiene
PDC/H_2O
HOOC
Dihydroarteannuic acid

FIG. 15

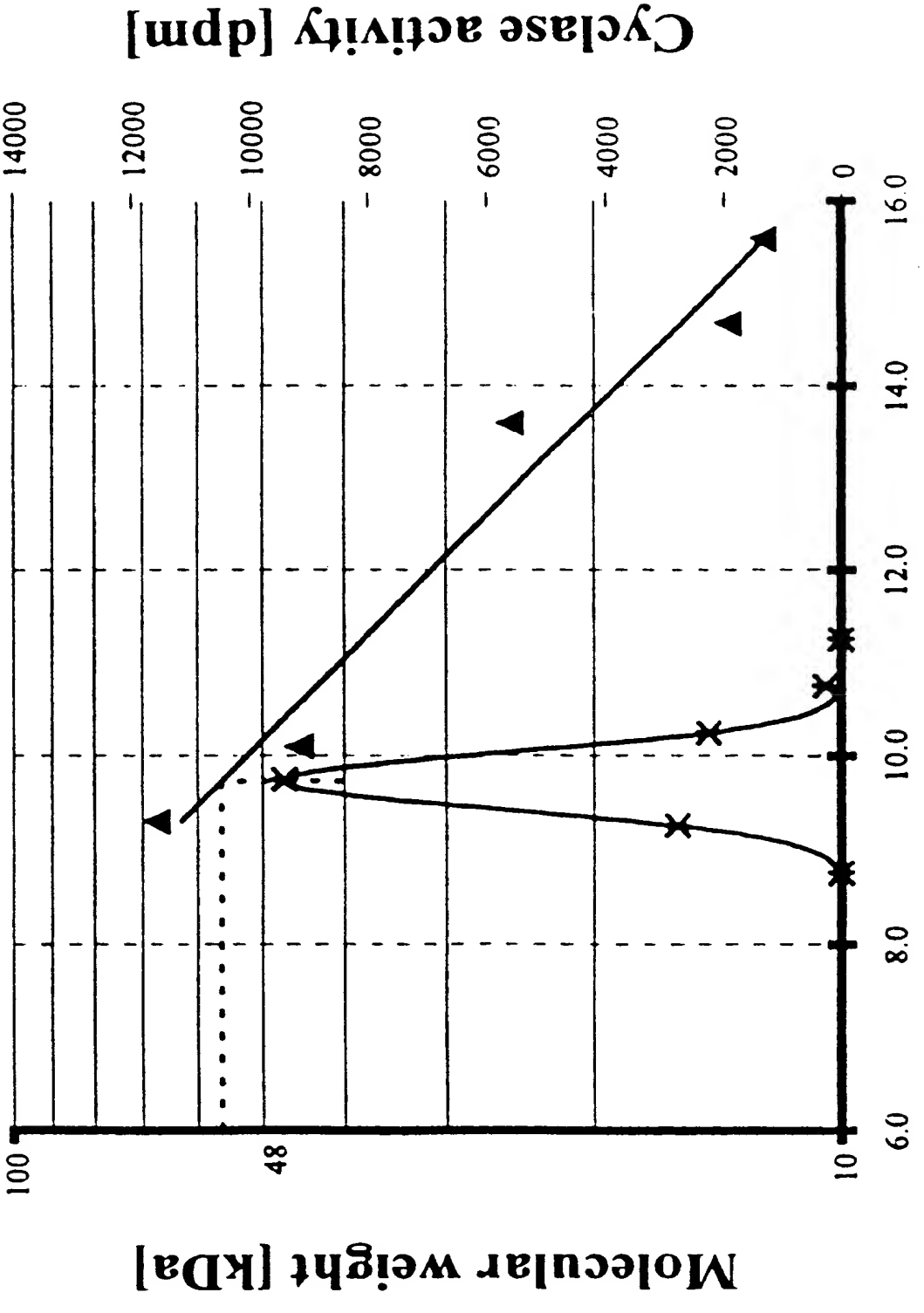

Cyclase activity [dpm]
14000
12000
10000
8000
6000
4000
2000
0
16.0
14.0
12.0
10.0
8.0
6.0
Elution volume [ml]
Molecular weight [kDa]
100
48
10

FIG. 16